

21 CFAL. However, excessive CFAL beyond the optimized dose inhibited M.A. removal
22 due to the hydrolysis and declining of molecular weight of chitosan that weakened the
23 bridging-netting property, where the surface charge reversal happened within a narrow
24 dosage range and the removal-dosage curve became parabola. The pH and
25 environmentally sensitive metal residuals in the algal solution were not significantly
26 affected by the adding of optimized dosage of CAFL-chitosan. The study provides a
27 possible way for HABs control using the cheap material of CFA. Further studies are
28 needed to check the potential influence of leachable metals and persistent organic
29 pollutants (pops) in CFA under a wide range of environmental condition.

30 **Key words**

31 Harmful algal blooms; *Microcystis Aeruginosa*; Flocculation; Chitosan; Coal fly ash.

32 **1. Introduction**

33 Harmful algal blooms (HABs) and lake eutrophication have been intensively studied
34 due to their threats to aquatic organisms, human health, costal aesthetics and
35 aquacultures (Gan et al. 2010, Thornton et al. 2013). Many approaches have been
36 tested to control the nutrient fluxes to the receiving water bodies including internal
37 and external loading management (Huser 2012, Sondergaard et al. 2002, Spears et al.
38 2013). However, in cases where nutrient management is not economically feasible or
39 the results obtained are unsatisfactory, additional strategies are needed to reinforce the

40 recovery such as algae harvesting (Chen et al. 2012), filtrations (Yadidia et al. 1977),
41 fish stocking (Jeppesen et al. 2012) and algicides (Garcia-Villada et al. 2004).
42 Aluminum and iron (Al/Fe) salts are widely used as geo-engineering materials for
43 P-sorption in eutrophic water. In addition, the aluminum and iron salts can be used as
44 flocculant because their hydrolysis products can overcome the electrostatic
45 stabilization of algal cells and promote flocs formation (Gonzalez-Torres et al. 2014).
46 Effective precipitation is generally obtained by Al/Fe salts when a ballast is included
47 (Pan et al. 2011a). Flocculation can be a welcome techniques combined with the
48 nutrient control methods for eutrophication restoration, which can improve the water
49 clarity and trigger submerged macrophyte restoration in shallow waters (Pei et al.
50 2014, Sun et al. 2013). However, the possible accumulation of Al in aquatic food
51 chain may pose risks to human health such as Alzheimer's disease (Kawahara and
52 Kato-Negishi 2011).

53 In recent years, efforts have been made on utilization of natural polymers as
54 flocculants such as chitosan (Li and Pan 2013, Pan et al. 2011a, Zou et al., 2006)
55 which may be biodegradable and less accumulated in aquatic food chain (Wang et al.
56 2015). Chitosan enhances HABs removal for local soil materials via charge
57 neutralization and bridging-netting effect (Li et al. 2015, Zou et al. 2006), however,
58 the algal removal rate may decline due to the folding of chitosan molecular chain in
59 high ionic strength and alkalinity environment (Pan et al. 2011a).

60 Commercial inorganic flocculants have been tested to improve the flocculation

61 efficiency of chitosan. Chitosan combined with poly aluminum chloride (PAC) can
62 turn local soils into effective flocculants. Over 90% of algal cells were removed using
63 10 mg/L PAC and 10 mg/L chitosan (Pan et al. 2011a). The PAC facilitates formation
64 of small flocs which are linked by chitosan into flocs 40% larger than using PAC
65 alone (Pan et al. 2011a). Coal fly ash (CFA) contains 25-30% Al_2O_3 and 6-15% Fe_2O_3
66 (Ahmaruzzaman 2010), which may potentially be a raw material for flocculation.
67 Several studies report that CFA based flocculants prepared from acid or alkaline
68 leachate of CFA are effective alternatives to commercial inorganic flocculants for
69 water purification (Fan et al. 2005, Yan et al. 2012). The flocculants derived from
70 CFA may have the potential to enhance the flocculation ability of chitosan. Besides,
71 CFA is a fine textured material and easily accessible in many cities which can
72 potentially accelerate flocs sedimentation by adding frame and weight to the flocs. So
73 far, few studies are seen on HABs removal using CFA and little is known on the
74 effects of using chitosan and Al/Fe in CFA on algae flocculation.

75 In this study, hydrochloric acid was used to extract Al/Fe in CFA. Chitosan was
76 modified by the leachate of CFA (CFAL) to prepare a compound flocculant
77 (CFAL-Chitosan) for M.A. flocculation. It is hypothesized that the Al and Fe in CFAL
78 can interacted with chitosan and form a compound flocculant which may enhance the
79 algal removal ability of chitosan. We evaluated the flocculation efficiency of the
80 compound flocculant via dosage effect on removal efficiency, surface charge, floc size
81 and stability. The FT-IR and molecular weight analysis were conducted to elucidate

82 the flocculation mechanisms. The objective of the study is to find a new method for
83 HABs control using chitosan and ways for CFA recycling.

84 **2. Materials and methods**

85 **2.1 Algal species and culture**

86 The *Microcystis aeruginosa* cell (M.A., FACHB-469) was obtained from the
87 Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB)
88 Chinese Academy of Sciences, and cultured in BG11 medium under controlled
89 conditions. Before autoclaving, the BG11 growth medium was adjusted to pH 8.0
90 using 0.5 mol/L NaOH or 0.5 mol/L HCl. The algae batch culture with initial density
91 of 1.23×10^8 cells/L was held in a 10 L glass vessel and kept at $25 \pm 1^\circ\text{C}$ under
92 2000-3000 lx of white fluorescent light on a 12 h light and 12 h darkness regime in an
93 illuminating incubator (LRH-250-G, Guangdong Medical Apparatus Co.Ltd., China).
94 Continuous aeration was supplied during the algae growth phase. The *M. aeruginosa*
95 cells under this condition were dispersed single cells (Li and Pan 2013).

96 **2.2 CFA and CFAL-Chitosan**

97 CFA was collected in a power plant in Datong City (Shanxi province, China). The
98 CFA was washed with deionized water three times, dried at 105°C , then sieved
99 through 180 mesh before use ($<90\mu\text{m}$, pre-treated CFA). The pre-treated CFA was
100 characterized by the X-ray fluorescence (XRF-1800, Shimadzu, Japan) and X-ray

101 Diffraction (X'Pert Pro MPD X-ray Diffractometer, Philips, Netherlands). The
102 Toxicity Characterization Leaching Procedures (TCLP, see Supplementary materials)
103 were carried out to determine the metal mobility of pre-treated CFA (USEPA 1994).
104 Leachates from three different extraction fluids (pH 2.88, 4.93 and 7.50) were
105 analyzed according to Inductively Coupled Plasma Emission Spectrometry (ICP-OES;
106 Optima 8300, PerkinElmer, USA).

107 Pre-treated CFA was used in two ways in this study. The 100 mg/L of pre-treated CFA
108 was utilized directly in the flocculation experiments and acted as ballast to assist
109 sedimentation processes. Besides, the leachate of pre-treated CFA (CFAL) was
110 obtained using hydrochloric acid and used for chitosan modification. The leaching
111 protocol was optimized through a preliminary test and set as 0.55 mol/L of
112 hydrochloric acid, solid/liquid ratio of 1 g:5 mL, leaching time of 24 h under 25°C at
113 agitation rate of 180 rpm in an oscillation incubator (HZQ-F160, HDL Electronic
114 Technology Development Co., LTD, China). The CFAL was separated from the
115 insoluble particles by 0.45 µm filter membrane. The metal concentrations in the CFAL
116 were measured by ICP-OES (Optima 8300, PerkinElmer, USA).

117 The chitosan powder was purchased from Qingdao Yunzhou Biochemistry CO.,LTD
118 which originates from crab shells. Four CFAL-Chitosan stock solutions were prepared
119 as algae flocculants, denoted as F-0, F-12, F-20 and F-40. The F-0 was prepared by
120 adding 0.5 g chitosan in 100 mL of 0.09 M acetic acid. Different volumes of CFAL (6,
121 10 and 20 mL) were diluted to 100 mL and 0.5 g chitosan was added to the dilutions

122 described above to prepare F-12, F-20 and F-40, respectively. The CFAL/Chitosan
123 ratio for F-0, F-12, F-20 and F-40 was 0 mL:1 g, 12 mL:1 g, 20 mL:1 g and 40 mL:1
124 g, respectively. The CFAL-Chitosan stock solutions were freshly made and diluted ten
125 times before use.

126 **2.3 Molecular weight and component analysis**

127 The molecular weight (M_v) of CFAL-Chitosan was obtained from the intrinsic
128 viscosity using Mark-Houwink-Sakurada equation reported before (Wang et al. 1991).
129 The intrinsic viscosity was determined using 0.2 M acetic acid/0.1 M sodium acetate
130 with Ubbelohde viscometer (Supplementary Materials, Intrinsic viscosity). The
131 viscosity of CFAL-Chitosan stock solution was quantified by rotational viscometer
132 (NDJ-1, Shanghai Yueping Scientific Instrument co., LTD, China).

133 The CFAL-Chitosan were dried and mixed with KBr in ratio of 1 mg: 100 mg for
134 FT-IR test (Nicolet 8700, Thermo Fisher, USA). The total Al and Fe in the
135 CFAL-Chitosan (F-12, F-20, and F-40) were measured by ICP-OES (Optima 8300,
136 PerkinElmer, USA). The Al bonded with chitosan (chitosan-Al) was separated by Al
137 fraction procedure (Vanbenschoten and Edzwald, 1990) and quantified by ICP-OES
138 (Optima 8300, PerkinElmer, USA). The free Fe was measured by polarograph (797
139 VA Computrace, Metrohm, Switzerland) and the Fe bonded with chitosan
140 (chitosan-Fe) was calculated as the subtraction of free Fe from the total Fe.

141 **2.4 Algae flocculation**

142 Flocculation experiments were set up in a jar test apparatus (ZR3-6, Zhongrun Water
143 Industry Technology Development Co., Ltd., China). Algal cells in the mid- to
144 late-exponential growth phase (Chen et al. 2004) were used and the cell concentration
145 was $4.15\text{-}4.23 \times 10^9$ cells/L in the flocculation experiments. The algal solution was
146 adjusted to pH 8.0 either by 0.5 mol/L NaOH or HCl before flocculation and 200 mL
147 of algal solution was transferred to 300 mL beaker for flocculation. In all flocculation
148 experiments, pre-treated CFA of 100 mg/L was added to the algal solution to assist
149 floc sedimentation. CFAL-Chitosan of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.0 and 6.0
150 mg/L (in terms of chitosan concentration) were added and the control was conducted
151 without adding any flocculants. The stirring process was 200 rpm for 1 min, 120 rpm
152 for 2 min, 40 rpm for 10 min. Samples (2 mL) from 2 cm below water surface were
153 collected after sedimentation for 0, 2, 5, 10, 20, 30, 40, 50 and 60 min for cell
154 counting. The removal rate was calculated as (initial cell concentration–sample cell
155 concentration)/initial cell concentration $\times 100\%$. The cells were firstly fixed with
156 Lugol solution (1% final conc.) and enumerated using a hemocytometer under
157 microscope (Axioskop 2 mot plus, Carl ZEISS, Germany). The zeta potential was
158 measured by Zetasizer 2000 (Malvern Co. UK). The floc growth during the
159 flocculation process was monitored by a laser particle size analyzer (Mastersizer 2000,
160 Malvern Co. UK). Samples were sent into the analyzer and back to the jar by a
161 peristaltic pump (BT00-300M, Baoding Longer Percision Pump Co. Ltd., China) with

162 a flow rate of 35 mL/min. The metal residuals including Al, As, Cr, Cd, Ba and Mn
163 after flocculation were quantified with ICP-OES (Optima 8300, PerkinElmer, USA).
164 The pH values were recorded before and after flocculation. The flocculation tests
165 were operated in triplicate and the results were presented as mean values.

166 **2.5 Floc stability**

167 Different shear force was applied to the flocs following the slow stirring process by
168 increasing the stirring speed to 75, 100, 150, 200 and 250 rpm for another 20 min. The
169 corresponding velocity gradient (G) values were 28.1, 41.3, 71.3, 105.0 and 141.7 s^{-1} ,
170 respectively. The dynamic flocs size was recorded as $d_{0.5}$ during the stirring process.
171 Referring to the empirical equation (Shi et al. 2015), the broken floc size was plotted
172 against the average velocity gradient in a log-log scale and the slope of the curve (γ) is
173 the main factor to quantify floc stability.

$$174 \quad \log d = \log C - \gamma \log G$$

175 where d is the median floc diameter ($d_{0.5}$) after breakage, μm ; C is the floc strength
176 co-efficient; γ is the stable floc exponent and G is the average velocity gradient, s^{-1} .

177 **3. Results**

178 **3.1 Characteristics of CFA and CFAL-Chitosan**

179 The pre-treated CFA used in this study mainly consisted of SiO_2 , Al_2O_3 , and Fe_2O_3
180 (Table S1). The XRD showed the presence of quartz (SiO_2), mullite ($3\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$),

181 hematite (Fe_2O_3) and corundum (Al_2O_3) in pre-treated CFA (Fig.S1). The metal ions
182 leached from the pre-treated CFA were more evident under acid conditions ($\text{pH}=2.88$)
183 but less concerned when pH was 7.5 (Table 1). The total Al and Fe in CFAL-Chitosan
184 increased with the increasing ratio of CFAL/Chitosan and the chitosan-Al and -Fe
185 were detected in CFAL-Chitosan (Fig.1).

186 Table 1 is here.

187 Fig.1 is here.

188 The molecular weight (M_v) of CFAL-Chitosan was calculated from the intrinsic
189 viscosity. The M_v of chitosan without CFAL (CFAL/Chitosan 0:1) was 682 kDa and
190 similar to that of chitosan powder. Both M_v and viscosity of CFAL-Chitosan
191 decreased as CFAL/Chitosan ratio increased. When CFAL/Chitosan ratio was 40:1,
192 the M_v and viscosity decreased 21.3% and 63.5% respectively compared to chitosan
193 without CFAL.

194 Fig.2 is here.

195 The chitosan powder and chitosan without CFAL (CFAL/Chitosan 0:1) exhibited
196 similar FT-IR spectra (Fig.3). A broad adsorption band around 3417 cm^{-1}
197 corresponded to the overlap of OH and NH_2 stretching vibration and peak at 2900
198 cm^{-1} was attributed to the stretching of CH (Ng et al. 2012). Band around 1650 cm^{-1}
199 referred to the amide I group, and peak at 1596 and 1561 cm^{-1} was the band of amide
200 II (Ng et al. 2012). The aliphatic OH band, acetal and glycosidic linkage were
201 associated with peaks at 1423 , 1154 - 1030 and 898 cm^{-1} , respectively (Ng et al. 2012,

202 Wang et al. 2011). The spectrum of chitosan with CFAL (F-12, F-20, and F-40 in
203 Fig.3) showed different characteristics from chitosan without CFAL. Band at 3417
204 cm^{-1} and amide I group shifted to lower wavenumber. The band of amide II and
205 aliphatic OH extinguished, however, a new band emerged around 1500 cm^{-1} .

206 Fig.3 is here.

207 **3.2 Dosage effect of CFAL–Chitosan**

208 For chitosan without CFAL, the M.A. removal reached to the peak of $81.6\pm 1.9\%$ at 3
209 mg/L then decreased significantly when chitosan dosage exceeded 3 mg/L (F-0 in
210 Fig.4). When the CFAL/Chitosan ratio was increased to 12:1, the maximum removal
211 rate plateaued at $98.2\pm 1.5\%$ at 3 mg/L and remained stable until the dosage increased
212 to 6 mg/L (F-12 in Fig.4). Removal rate of $95.0\pm 1.5\%$ was found at 3.5 mg/L for
213 CFAL/Chitosan ratio of 20:1 (F-20 in Fig.4). When the CFAL/Chitosan ratio further
214 increasing to 40:1 (F-40), the algae removal reached to the peak of $76.5\pm 2.8\%$ at 2
215 mg/L, which was quickly reduced beyond the optimal dosage of 2 mg/L (F-40 in
216 Fig.4). The zeta potential of M.A. flocs increased as CFAL-Chitosan was added to the
217 algal solution. For F-0 and F-40, the charge of M.A. flocs reversed at 5 mg/L and 3.5
218 mg/L, respectively. While the charge reversals were not observed for both F-12 and
219 F-20 bellow the dosage of 6 mg/L. According to the dosage-efficiency curves, the
220 CFAL-Chitosan dosage was set as 3 mg/L for the floc growth, flocculation kinetic and
221 floc stability experiments.

222

Fig.4 is here.

223 **3.3 Floc growth and flocculation kinetics**

224 Using pre-treated CFA up to 100 mg/L did not promote M.A. aggregation and the
225 removal efficiency was nearly zero (Fig.5 & 6). For chitosan without CFAL, the
226 growth of flocs plateaued at 12 minutes with floc size of approx. 560 μm (F-0 in
227 Fig.5). After sedimentation for 5 min, the removal rate of F-0 reached 79.7% and kept
228 a stable trend as time increased (F-0 in Fig.6). When CFAL/Chitosan ratio increased
229 to 12:1 and 20:1, the floc size increased to 750 μm (F-12 & F-20 in Fig.5), but F-12
230 exhibited a faster growth rate. The removal efficiency of F-12 reached 97.2% within 2
231 min and remained stable, while 87.5% of algal cells were removed for F-20 after
232 sedimentation for 60 min (Fig.6). When CFAL/Chitosan ratio increased to 40:1, the
233 floc size (380 μm) decreased compared to F-0, F-12 and F-20 and a lower removal
234 rate of 72.8% was achieved at 60 min (Fig.5 & 6).

235

Fig.5 is here.

236

Fig.6 is here.

237 **3.4 Floc stability**

238 The stability of algae flocs at 3 mg/L CFAL-Chitosan was tested by measuring the
239 floc size changes after applying a shear force (Shi et al. 2015). The stable floc
240 exponent (γ) is a quantitative measurement of floc stability. When CFAL/Chitosan
241 ratio was 12, the γ of flocs was 0.39, lower than chitosan without CFAL (0.49)

242 indicating that the floc stability was improved (Fig.7). However, when excessive
243 CFAL was added (F40), the floc stability decreased compared to CFAL-Chitosan
244 (F-12) (Fig.7).

245 Fig.7 is here.

246 **4. Discussion**

247 **4.1 The M.A. removal by chitosan without CFAL**

248 The zeta potential of M.A. flocs was -34.8 mv when pre-treated CFA alone (100 mg/L)
249 was added and the algal cells were not removed due to the electrostatic repulsion
250 (Fig.4 & 6). When chitosan without CFAL (CFAL/Chitosan ratio =0:1) was added at 3
251 mg/L, the removal rate reached to the peak of $81.4\pm 1.9\%$ and the zeta potential
252 increased from -34.8 to -15.4 mv, indicating the electrostatic repulsion was reduced,
253 which may due to the attraction between amine groups of chitosan and algal cells (F-0
254 in Fig.4). Chitosan is a linear biopolymer with high molecular weight (682 kDa, Fig.2)
255 and has a long polymer chain structure (Li et al. 2013). The flocs of large size were
256 formed (560 μm) through electrostatic attraction and bridging-netting function by the
257 long polymer chain of chitosan (F-0 in Fig.5). However, 18.6% of algae cells were not
258 removed since the algae flocs were not sufficiently neutralized with zeta potential far
259 below zero (-15.4 mv) at the optimized dosage of chitosan without CFAL (3 mg/L)
260 (Li et al. 2015). Besides, the M.A. flocculation was not stable and declined
261 significantly at 5 mg/L due to the reversal of the zeta potential (+3.4 mv) and

262 re-stabalization of algal flocs (F-0 in Fig.4).

263 **4.2 The M.A. removal by chitosan with CFAL**

264 Although CFAL alone was not effective in M.A. removal (Table S2), it enhanced M.A.
265 flocculation of chitosan (F-12, F-20, Fig.4). The removal rate of F-12 and F-20
266 reached over 95% at 3 and 3.5 mg/L, respectively and was higher than chitosan
267 without CFAL (Fig.4). Moreover, the floc of F-12 were 34% larger and more stable
268 and sunk faster together with a ballast than chitosan without CFAL (Fig.5, 6 & 7).
269 When the flocculant dosage was beyond the optimized dosage, the zeta potential of
270 algal flocs using chitosan with CFAL (F-12, F-20) stayed near zero and the algal
271 removal efficiency plateaued over 90%. While for chitosan without CFAL (F-0), the
272 removal efficiency peaked at 3 mg/L with a lower removal efficiency than
273 CFAL-Chitosan (F-12, F-20) and significantly decreased due to the fast reversal of
274 zeta potential at higher dosage (5~6 mg/L, Fig.4). This indicated that CFAL-Chitosan
275 can maintain a better algal removal rate over wide dosage range due to improved
276 property on charge neutralization. The component analysis confirmed the formation of
277 chitosan-Al and -Fe in the prepared flocculants (Fig.1). Compared with the FT-IR
278 spectrum of chitosan without CFAL (CFAL/Chitosan ratio=0:1), the amide II and
279 aliphatic OH groups disappeared when chitosan was modified by CFAL (F-12, F-20,
280 F-40 in Fig.3). A distinct band emerged at 1500 cm^{-1} which could potentially be the
281 characteristic of Al-NH₂ or Fe-NH₂ (Himmel et al. 2000, Wang et al. 2011). It
282 indicated that the OH and NH₂ of chitosan might chelate with Al and Fe in CFAL.

283 The free Al/Fe in CFAL-Chitosan may also contribute to enhancing the charge
284 neutralization of chitosan and it requires further studies to explore the functions of
285 chitosan-Al and -Fe.

286 For F-12, when the dosage was higher than 3 mg/L, the electrostatic repulsion
287 between M.A. flocs kept low and the M.A. removal remained over 90% (Fig.4).
288 However, when the CFAL/Chitosan ratio increased to 40:1, sharp decline of algal
289 removal occurred again at 3.5 mg/L due to reversed charge (+2.6 mv). This indicated
290 that excessively increasing the CFAL/Chitosan ratio may result in faster reversal of
291 algal charge and narrow the dosage range for good algal removal. The flocs formed at
292 F-40 was less stable with higher γ value under the conditions tested compared to F-12.
293 Effective M.A. flocculation was generally obtained at the dosage where the zeta
294 potential of algal flocs was near zero. In this study, moderate amount of CFAL
295 (CFAL/Chitosan ratio 12:1) optimized the charge neutralization of chitosan and a
296 wide dosage range for effective M.A. removal was obtained.

297 The long chain structure of chitosan is largely responsible for the bridging-netting
298 property which is positively related to the molecular weight (Li et al. 2013). When
299 chitosan was modified by CFAL, the molecular weight (M_v) of CFAL-Chitosan
300 decreased (Fig.2), indicating that the long chain structure of chitosan was adversely
301 influenced and the bridging-netting ability was weakened by the over dosed CFAL.
302 The hydrochloride acid in CFAL may trigger the hydrolysis of chitosan molecules
303 (Vårum et al. 2001). The floc size and stability of chitosan modified by CFAL

304 decreased and flocs sedimentation became slower under CFAL/Chitosan ratio of 40:1,
305 which supported the weakening of bridging-netting effect (Fig.5 & 6).

306 **4.3 Flocculation materials and methodology**

307 Previous studies have revealed that particles with the right size can enhance the
308 collision frequency and add frame to the flocs to accelerate sedimentation (Chen and
309 Pan 2012, Li and Pan 2013, Pan et al. 2006, Park et al. 2013). In this study, when
310 using CFAL-Chitosan (F-12) at 3 mg/L without pre-treated CFA, the removal
311 efficiency of algal cells was $89.6\pm 0.6\%$. This was about 8% lower than 3 mg/L
312 CFAL-Chitosan (F-12) with 100 mg/L of pre-treated CFA. Pre-treated CFA, an
313 alternative ballast material to local soil, facilitated algal removal when used with
314 CFAL-Chitosan (F-12 in Fig.4). As a solid waste, the ecological safety of CFA
315 including CFA particles and CFAL is the prerequisite for its application in natural
316 waters. Since the heavy metal ions such as Mn and Ba (Table S3) were detected in
317 CFAL, the dosage of CFAL used in chitosan modification should be carefully
318 optimized which was closely related to the amount of heavy metals ions introduced to
319 the algal solution. Although the metal mobility in pre-treated CFA under alkaline
320 conditions was low (pH=7.50) and within the allowable limits of USEPA standard for
321 hazardous materials (1994), it may be a concern under acid conditions (Table 1). CFA
322 may also contain persistent organic pollutants such as PAH and dioxin. The
323 availability of these pollutants in CFA under wide environmental conditions needs
324 further investigation. Moreover, CFA composition varies from coal types and

325 combustion processes. CFA screening is essential before it can be used for HAB
326 control.

327 The pH and metal residuals in algal solution before and after flocculation were not
328 significantly influenced (Table S4) at the conditions tested here. Hydrochloric acid is
329 a frequently used extracting agent to prepare CFA based flocculants (Choo et al. 2014,
330 Yan et al. 2012). In this study, hydrochloric acid can extract Al/Fe in CFA which
331 improve the charge neutralization for chitosan. However, concentrated hydrochloric
332 acid can result in the hydrolysis and decrease of molecular weight of chitosan, which
333 inhibits the bridging-netting ability (Fig.2). For CFAL/Chitosan ratio of 40:1, the M.A.
334 removal was $73.6 \pm 3.6\%$ at 3 mg/L although the M.A. cells were neutralized with zeta
335 potential near zero (-2.8 mv, Fig.4). During the preparation of CFAL-Chitosan, CFAL
336 was diluted suggesting that the acid concentration used for CFA leaching can be
337 reduced in practical application to alleviate the negative impacts on chitosan structure.
338 There was a balance between the charge neutralization enhancement and structural
339 influence of chitosan when modified by CFAL. It is likely that the M.A. removal can
340 be potentially improved by screening mild extracting agents which not only extract
341 Al/Fe but also maintain the chitosan structure.

342 **4.4 Environmental implications**

343 In the past decades, efforts have been made to reduce the external loading via
344 improving environmental standards such as wastewater treatment and agriculture, and
345 internal loading such as adding P-sorption materials and sediment dredging

346 (Drabkova and Marsalek 2007). However, many additional physical, chemical and
347 biological methods have been developed to reinforce recovery when obtained results
348 are unsatisfactory. Flocculation can quickly remove the suspended algal cells down to
349 the sediments and improve water transparency which provides favorable conditions
350 for photosynthesis and/or submerged macrophytes restoration in shallow waters
351 (Bakker et al. 2013). The usage of CFAL-Chitosan as algal flocculant may have
352 positive side-effects such as killing the settled algal cells since the breakdown
353 products of chitosan are suspected to have antibacterial activities (Wisniewska-Wrona
354 et al 2007) but the latter requires further studies. In addition, using pre-treated CFA as
355 alternative ballast to replace local soil has several advantages in some cases. Firstly,
356 CFA is produced in large quantity and convenient to access for places with thermal
357 power plant. While local soils may be not easily available especially in developed
358 urban areas (prohibited by urban planning/regulations). Secondly, the CFA is a fine
359 textured material which easily collides with algal cells (Han & Kim 2001). The
360 pre-treatment of CFA described in this study such as washing, drying and sieving may
361 not be needed in practical application and CFA may be used directly without
362 processing after careful check of heavy metal and persistent organic pollutants. While
363 the handling cost of local soils could be substantial when using labor for digging,
364 grinding, sieving, and washing. Thirdly, CFA is a solid waste of low value and the
365 cost of CFA disposal may be a burden for the producing factories. While local soils
366 are important resources for urban planning, landscape conservation and agriculture. In

367 cases where CFA is available and local soil is prohibited to be collected at large scale,
368 CFAL-chitosan method may provide a possibility to utilize CFA for HAB control.

369 Controlled lab stirring condition is essential for repeating and revealing the
370 mechanisms of algal flocculation. However, in the field, the flocculation behavior
371 could be influenced by many factors such as the type of algae (single or colonial cells),
372 pH, salinity, vertical and horizontal mixing of water etc. Preliminary jar tests are
373 required before field application. Moreover, the flocs were prone to break under
374 turbulent conditions (Fig.S2) and this can be a problem in shallow lakes where
375 wind-oriented turbulence is inevitable. The degradation of algae may damage the cell
376 membrane integrity which might stimulate the release of microcystins and consume
377 dissolved oxygen. In addition, accumulation of algal flocs on lake sediments could
378 influence the redox condition of the sediment and thereby influence pollutant fluxes
379 from sediment to overlying water such as nutrients fluxes. It was reported that
380 capping materials may be helpful in solving these problems (Pan et al. 2012). The
381 microorganism modified capping materials could be effective for decomposing
382 microcystins released from the broken *M. aeruginosa* (Li and Pan 2015). Capping
383 materials loaded with oxygen nanobubbles may improve the hypoxia condition near
384 the sediment and alleviate pollutants released from sediments (Pan and Yang 2012). In
385 some cases, it is possible to utilize the settled flocs as fertilizer for the restoration of
386 submerged macrophytes (Pan et al. 2012, Pan et al. 2011b). The control of adverse
387 effects after algal flocculation is a very complex issue and the possibility to

388 manipulate them using geo-engineering methods needs further studies.

389 The use of non-biodegradable chemicals such as FeCl_3 and PAC, or alum may pose
390 risks to human health such as Alzheimer's disease through bio-accumulation
391 (Kawahara and Kato-Negishi 2011). In this study, at the optimized dosage of
392 CFAL-Chitosan, the calculated Al dosage was 0.02 mg/L (F-12, 3 mg/L) and
393 significantly lower compared to the effective dosage reported in other studies
394 (Gonzalez-Torres et al. 2014, Paul et al. 2008). Introducing small amount of CFAL
395 can improve the flocculation efficiency of chitosan and CFAL-Chitosan to some
396 extent decreased the use of bulk chemicals. Table S5 estimated the cost of several
397 methods for HABs control. To achieve removal rate over 90%, the cost of
398 CFAL-Chitosan is 0.07 US\$/m³, which is lower than the PAC-Chitosan (0.23 US\$/m³)
399 and *Moringa oleifera*-Chitosan (MO-Chitosan, 5.19 US\$/m³) (Li and Pan 2013, Pan
400 et al. 2011a). In further studies, it is possible to reduce the cost by screening cheap
401 biopolymers as chitosan alternatives such as cationic starch and larch tannin (Shi et al.
402 2015, Wang et al. 2013).

403 **5. Conclusion**

404 In this study, we developed a compound flocculant using coal fly ash leachate (CFAL)
405 modified chitosan for *Microcystis aeruginosa* (M.A.) flocculation. It was found that
406 the CFAL enhanced flocculation ability of chitosan for M.A. removal at
407 CFAL/Chitosan ratio of 12:1 and good algal removal rate remained in a wide dosage
408 range due to the improvement of charge neutralization property. The algal flocs of

409 CFAL-Chitosan were larger and denser than chitosan without CFAL. However, when
410 CFAL/Chitosan ratio was increased beyond the optimal, surplus of CFAL inhibited
411 the M.A. removal due to the hydrolysis and declining of molecular weight of chitosan
412 which impaired the bridging-netting property. New mild extracting methods should be
413 studied in the future which not only extract Al/Fe in CFA but also maintain the
414 chitosan structure at the same time. CFA combined with CFAL-Chitosan can be a
415 possible economical way for HABs mitigation owing to its easy availability and
416 pretreatment processes. Further studies are needed to check the potential influence of
417 leachable metals and persistent organic pollutants (pops) in CFA under a wide range
418 of environmental condition.

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563

564 **FIGURE CAPTIONS**

565 **Fig.1**-The Al and Fe in CFAL-Chitosan, F-12: CFAL/Chitosan ratio 12:1, F-20:
566 CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1.

567

568 **Fig.2**-The molecular weight (kDa) and viscosity (cps) of chitosan powder and
569 CFAL-Chitosan, F-0: CFAL/Chitosan ratio 0:1, F-12: CFAL/Chitosan ratio 12:1, F-20:
570 CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1.

571

572 **Fig.3**-The FT-IR spectra of chitosan powder and CFAL-Chitosan, a: F-12
573 CFAL/Chitosan 12:1, b: F-20 CFAL/Chitosan 20:1, c: F-40 CFAL/Chitosan 40:1, d:
574 F-0 CFAL/Chitosan 0:1, e: chitosan powder.

575

576 **Fig.4**-Algal removal efficiency and zeta potential of M.A. flocs as function of
577 CFAL-Chitosan dosage. F-0: CFAL/Chitosan ratio 0:1, F-12: CFAL/Chitosan ratio
578 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1, initial pH 8.0,
579 pre-treated CFA concentration 100 mg/L.

580

581 **Fig.5**-The dynamic floc size of M.A. cells after addition of 3 mg/L CFAL-Chitosan,
582 initial pH 8.0, pre-treated CFA concentration 100 mg/L, F-0: CFAL/Chitosan ratio 0:1,
583 F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40:
584 CFAL/Chitosan ratio 40:1.

585

586 **Fig.6**-The flocculation kinetics of M.A. cells after addition of 3 mg/L CFAL-Chitosan,
587 initial pH 8.0, and pre-treated CFA concentration 100 mg/L, F-0: CFAL/Chitosan ratio
588 0:1, F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40:
589 CFAL/Chitosan ratio 40:1.

590

591 **Fig.7**-Floc stability plots of CFAL-Chitosan at 3 mg/L (pre-treated CFA dosage, 100
592 mg/L, initial pH=8.0, Shear time, 16 min). F-0: CFAL/Chitosan ratio 0:1, F-12:
593 CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan
594 ratio 40:1.

595